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PREFACE

This ^{report} ~~paper~~, the second in the American Biophysics Research Laboratory's Monograph Series, is devoted to applications of the laser in the fields of the biological sciences. The first paper in this series was concerned with ~~electronic~~ microminiaturization and its potential uses in these same areas. Work on these papers was sponsored by the Office of Naval Research in order ^{and} to provide semitechnical descriptions of recent advances in the fields of engineering and physics so that biologists and biophysicists may acquaint themselves with new techniques and instrumentation which will prove useful to them in the pursuit of their research activities. In content, the papers are biologically oriented and areas which are technically involved, either in terms of physics or mathematics, are omitted. For those readers who desire to pursue the subjects presented in more technical detail, ^{an} extensive bibliography and references are included.

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I. INTRODUCTION

The field of quantum electronics has arisen from the application of the results of quantum mechanics to electronics. The first practical device to spring from this union was named the maser, a term which stands for Microwave Amplification by Stimulated Emission of Radiation. The discovery of the maser and its principles of operation is credited to C. Townes (Columbia University) and J. Weber (University of Maryland) in this country, and N. G. Basov and A. M. Prokhorov of the Soviet Union.^{1,2,3,4} With amplification of maser theory and the discovery of new techniques to produce maser action, there were developed two more devices which worked at much shorter wavelengths. These devices are called the iraser for Infrared Amplification by Stimulated Emission of Radiation, and the laser for Light Amplification by Stimulated Emission of Radiation. They are also known as the infrared maser and optical maser respectively.

The basic theory of operation (see Appendix I, Principles of Laser Operation) for all three devices is essentially the same. In this monograph, we are interested primarily in the properties of the optical maser which spans the frequency spectrum from the near infrared to the ultraviolet. These properties are monochromaticity (radiation of a single wavelength), a very high degree of spatial and temporal coherence (phase characteristics of a point source), and, as a consequence, very high energy densities

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1. Gordon, James P., Scientific American, 199, No. 6 P. 42 (1958).
 2. Gordon, J. P., H. J. Zeiger and C. H. Townes, Phys. Rev. 99 No. 4 P. 1264 (1955)
 3. Weber J., Rev. Mod. Physics, 31 No. 3 P. 681 (1959)
 4. Basov, N. G. and A. M. Prokhorov, Akademiya Narik, SSSR, Doklady, 101, No. 1, P. 47-49 (1955).

(intensity) at the point of impingement. A discussion of the uses which have been made and can be made of these properties in biological systems forms the basis for this monograph.

II. PRESENT USES OF LASERS IN BIOLOGY

A. PHOTOCOAGULATION USING LASER BEAMS

The only practical experiments reported in the literature to date⁵⁻⁹ have been those concerning photocoagulation of the retina and iris. Dr. Milton M. Zaret and his group at New York University have reported^{5,6,8} on experiments with pigmented and albino rabbit eyes. Technical considerations concerning the laser used (TRG Vire. I) were handled by members of the Technical Research Group, a commercial organization active in the laser field. The following is a brief description of the experimental technique and the results obtained.

A ruby laser was set up on an optical bench so that it was free to move vertically and horizontally for aiming purposes. The rabbit was then placed in a position such that the laser beam would impinge on the eye and focus on the retina after refraction. The laser produced pulses with an intensity of 0.1 joule/cm^2 with a .5 millisecond duration. One pulse was applied to the eye and then the eye was examined and photographed with an ophthalmoscope. The experiment was then repeated using albino rabbits. In another set of experiments, run concurrently, the laser beam was aimed at the iris of the eye.

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5. Zaret, Milton M., et al., New York University Medical Center (1961). ASTIA #AD264,393.
 6. Zaret, Milton M., et al., Abstract, J. Optic. Soc. Amer. 52:607 (1962).
 7. Koester, C. J., et al., Optic Soc. Amer. 52:607 (1962).
 8. Zaret, Milton M., et al., Science 134:1525 (1961).
 9. Zaret, Milton M., et al., Archives of Ophthalmology 69:131 (1963).

Examination of the rabbits' eyes revealed instantaneous thermal damage to both retina and iris after exposure to the laser pulse. This thermal damage spread slowly in a circle whose center was the main point of impingement. The retina became firmly attached to the choroid and pigmented epithelium was not present. The albino rabbits showed minimal lesions and vitreous bubbling.

In their most recent work⁹, Dr. Zaret's group used the same experimental setup but attenuation filters were inserted between the laser and the eye. Decreasing the amount of energy incident on the eye produced less thermal spreading and a smaller area of coagulation.

Dr. Koester's group at Columbia University has reported⁷ the use of the laser for photocoagulation of the human retina. The laser beam is reflected onto the patient's eye using a dichroic mirror so that the physician may observe the patient's eye by looking through the mirror.

B. USES OF LASERS IN MICROSURGERY

The laser beam represents a highly defined beam of high intensity monochromatic light. When this beam strikes solid matter, three phenomena may occur singly or in combination: (1) reflection; (2) refraction; and, (3) absorption. The latter may be subdivided into: (a) true absorption with an increase in the total energy of the system; and, (b) scattering. The true absorption of energy from the laser beam causes a temperature rise in the absorber. The temperature rise is not confined to the immediate area but spreads from the point of impact in a thermal wave. The extent of thermal spreading depends upon the heat conductivity of the absorber and the instantaneous temperature gradient between adjacent regions of the

absorber. The effect of thermal spreading has been shown in photocoagulation.

Appendix II shows that lasers may be used to illuminate or destroy single cells or portions thereof. The possibility of doing this was first put forth by C. Townes.¹⁰ In early 1963, a team at Technical Research Group, Inc. has demonstrated the physiological effects of laser beams on the cell level. Portions of the cell were shown to be damaged without causing irreversible effects to surrounding areas¹¹. This work should soon be published in Science.

The use of laser beams in microsurgery is very desirable since it provides a means of performing very definitive surgery without damaging surrounding tissues. The target cell area may be brought to a focus under a microscope. The same optics are then used to focus the laser beam on the target. Suitable optics could also be included for observation while the destruction is taking place. The laser beam could also be used to illuminate the material being viewed, providing the beam were highly attenuated. Such a technique would obviously be limited to regions which could be viewed directly.

A discussion of surgical techniques to be employed within the body without first exposing the area of interest using conventional surgical techniques, poses many difficulties since many of the parameters which are vital for an intelligent appraisal are relatively unknown. The most

10. Townes, C. H., Biophys. Jour., 2, No. 2, p. 325 (1962)

11. Personal communication to author by Charles A. Roth of Technical Research Group, Inc., March 20, 1963.

important of these is a quantitative knowledge of the absorption coefficient of the various tissues. The absorption coefficient of a substance represents the amount of energy removed from a beam of light in its passage through a unit thickness of the substance. Qualitatively, one finds that light having a wavelength of 6000 angstroms (red) incident upon the surface of the skin penetrates to the subcutaneous region. This being the case, practically no energy would be available to the deeper body organs and hence the technique would be useless except in the first few centimeters of body tissue. At wavelengths greater than 6000 angstroms (in the near infrared) deeper penetration is possible. Two approaches are possible: (1) experimenters may obtain lasers which operate at various frequencies and attempt their use in specific problems; or, (2) a program may be established to provide absorption spectra for various body tissues, such as fat, smooth muscle, or striated muscle. The latter approach would be the most desirable, even if it proves necessary to use a single laser and various tissues and combinations thereof, since the results would be quantitative in nature and would allow further experiments to be performed with some predicted degree of success. While all substances exhibit absorption maxima, it should be pointed out here that there are also regions of the absorption spectrum of a particular substance which exhibit absorption minima. These minima are referred to as windows. The location of these windows is vitally important since it would enable the laser beam to act on underlying tissues with a minimum absorption in the overlying one. An example of such a window is the transmissivity of sea water in the region between 4000 and 6000 angstroms which imparts the blue color to thick layers. It is

interesting to note here that Semi-Elements, Inc. has recently announced the development of a laser operating at 5460 angstroms.¹² Lasers at these frequencies are being developed for underwater communications and possible ranging instrumentation. The effect, if any, which marine organisms will have upon the attainment of these goals, and vice versa, must be taken into account in the design of any reliable undersea communications system.

One further area of experimentation is suggested by the work of Fry with focused ultrasonic waves.¹³ A portion of a cat's skull is removed and ultrasonic waves are focused at some depth in the underlying brain tissue causing its selective destruction. Postulating a minimal absorption by the surrounding brain tissue, it should be possible, using the proper wavelength, to destroy regions of the brain in a far more selective fashion, since the laser beam is capable of being focused to a much smaller spot size than the ultrasonic beam.

12. Corneretto, A., Electronic Design, P. 30, October 11, 1962.

13. Fry, W. J. et al., Ultrasound in Biology and Medicine, et. E. Kelly, Amer. Instit. Biol. Sci., Washington D.C., P. 110 (1957).

III. POTENTIAL APPLICATIONS OF LASER BEAMS¹⁴⁻¹⁷

The use of high concentrations of monochromatic light energy, such as can be derived from lasers, has certain spectacular research areas. Most interestingly, the recent development of ultraviolet lasers will permit the evaluation of the effects of ultraviolet light upon proteins, nucleic acids, fungi, and bacteria.

It has been known for many years that ultraviolet light has lethal effects on large numbers of bacteria, fungi, and viruses. The usual explanation given is that the absorption of the bacteria protein is highest in the region of 2650 Å. One of the greatest difficulties in the utilization of the phenomenon is that a large number of quanta of this particular frequency must be secured in order to destroy the species. Utilizing laser techniques, it is quite reasonable to suppose that remarkable effects may be secured upon these species. What would be most interesting would be the evaluation of the absorption spectra and effects upon proteins of various types and upon their constituent molecules, the amino acids, of various frequencies and intensities of ultraviolet and visible light at the high intensities available from lasers. This is especially so since it is quite possible

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14. Stuhlman, O., An Introduction to Biophysics, John Wiley & Sons, New York, Chapter IV (1943).
 15. Stacy, Ralph W. et al., Essentials of Biological and Medical Physics, McGraw-Hill, New York, Part V. (1955).
 16. Setlow, Richard B. and Ernest C. Pollard, Molecular Biophysics, Addison-Wesley, Reading, Mass., Chapters 6,7,8,9 (1962).
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now to focus the light energy upon small targets. Attempts can be made to affect long chain protein molecules by selectively bombarding them with very short wavelengths of laser energy. Since the wavelengths of laser light are now in the ultraviolet range and since the dimensions of the molecule approximate that of the wavelength of ultraviolet light, it is conceivable that specific bombardment of the protein molecule would be possible. This is particularly interesting in attempts to modify the protein molecule by utilizing laser energy. Each chemical bond has characteristic absorption frequencies. Selective protein chemical bonds can be broken by laser frequencies to effectuate unique protein modification.

Some further possibilities would be the application of laser energy to specific enzymes systems. If the technique proves effective, the biologist could demonstrate the modification or the destruction of the enzyme system by using laser energy. It is known that, because of the number of quanta which are necessary to inactivate single bacteria and even single virus or enzyme molecules, large quantities of light energy would have to be applied for this purpose. However, by utilization of the tremendous energy-densities available from single laser exposures, it becomes quite enticing to consider the possibilities of using lasers for the modification of viruses, bacteria, proteins and enzyme systems.

Certain direct effects of high intensity laser beams might be considered as well. Certainly the characteristics of the effects of laser beams on various suspensions in ocean waters might be of value. Thus, consideration can be given to the amount of absorption of ultraviolet, visible and infra-red light by sea water of various concentrations. The absorption of these

same energy bands by the small plankta which may be present in the water might also be interesting. The scattering of light energy by plankta should also be investigated. This is possible through the use of lasers because the amount of energy available in a laser beam is far greater than from any present technique. Additionally, the use of laser beams for the measurement of the movement of plankta and other underwater inhabitants is feasible since the coherency of the light, its short wavelength, and high stability permit the measurement of very small velocities. Thus, only by the use of a laser beam would it be possible to measure velocities of plankta, marine animals, tides, and other slowly moving objects beneath the ocean's surface. There is even a very reasonable possibility that marine creatures might be observed from the surface through the utilization of laser techniques. The extremely high energies available from laser beams would permit the singling out of large marine creatures by selective emission into sea water and the plotting of the return reflections. This is in effect, laser ranging utilizing the high energy-density of the laser beam. The laser beam might be used in this fashion to secure a photograph of the ocean from the surface down. This only becomes possible due to the high energy-density of laser emission.

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10. Nathan, Marshall I. et al; Stimulated Emission of Radiation from GaAs p-n Junctions; Applied Physics Letters, Volume 1, Number 3, Page 62.
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NOTE: Proc. IRE, 51, Number 1, January 1963, Entire Issue Devoted to
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APPENDIX I

PRINCIPLES OF LASER OPERATION

The quantum theory was first advanced by Max Planck to explain black-body radiation or radiation from a container whose walls are kept at a constant temperature. According to the quantum theory, an atom is visualized as being composed of stable energy states whose energies are given by the relation:

$$E_n = h f_n, n = 1, 2, 3, \dots \quad (\text{I-1})$$

where: h is Planck's constant (6.63×10^{-34} joule-sec) and f is the characteristic frequency of that energy state in cycles per second. Hence, energy is considered to be composed of small packets or quanta. The quantum of electromagnetic energy is called the photon.

If a system (whether atomic or molecular) can be characterized by two energy levels E_1 and E_2 , then from Equation (I-1) we have:

$$E_1 = h f_1 \quad (\text{I-2})$$

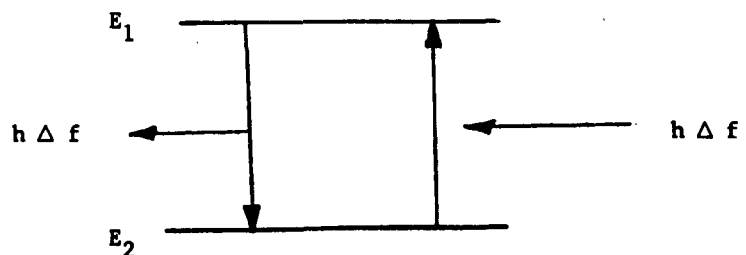
and

$$E_2 = h f_2 \quad (\text{I-3})$$

The difference in these energy states is given by

$$E_1 - E_2 = h (f_1 - f_2) = h \Delta f \quad (\text{I-4})$$

If $E_1 > E_2$ the system emits energy in the transition from level 1 to 2 and absorbs energy in the transition from level 2 to 1. The energy emitted or absorbed is given by $h \Delta f$. This can be represented by the following energy level diagram:



Although the remarks which follow are directed toward a system composed of single atoms, similar statements can be made regarding molecules.

The number of atoms occupying a given energy state is given by the Boltzmann energy distribution:

$$N = N_0 \exp (-E/kT) \quad (I-5)$$

Where:

N_0 = Total number of atomic systems

k = Boltzmann's constant (1.38×10^{-16} erg/deg)

T = absolute temperature.

For energy state E_1 we have

$$N_1 = N_0 \exp (-E_1/kT) \quad (I-6)$$

and for E_2

$$N_2 = N_0 \exp (-E_2/kT) \quad (I-7)$$

or

$$\frac{N_1}{N_2} = \exp [-(E_1 - E_2)/kT] \quad (I-8)$$

If, in equilibrium, $E_1 > E_2$, the lower energy state E_2 has the largest number of atoms or the largest "population". If, by some means, the population of energy level E_1 could be made greater than that of E_2 (population inversion), the concept of negative temperature will apply (as discussed in the literature). The creation of a population inversion is a prerequisite for laser action and is obtained by forcing energy into the system or "pumping", so that atoms in the lower energy state are raised to the higher. When the population returns to normal, photons having a frequency of Δf are given off.

Thus, we have an atomic system composed of discrete energy levels. When a photon or corpuscle of radiation of a given frequency enters such a system, one of three things may happen:

(1) If its frequency does not correspond to the difference in frequency between two energy levels in the system, the photon may pass on undisturbed;

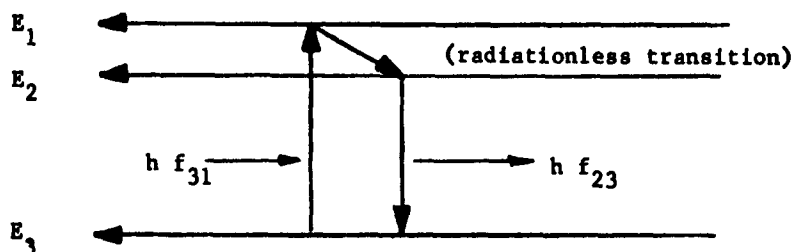
(2) If its frequency does correspond to the frequency difference between two energy states the photon may be absorbed causing a transition from the lower energy state to the higher;

(3) If its frequency does correspond to the frequency difference between the two energy states, the photon may stimulate a transition from the higher energy state to the lower, thus resulting in the production of another photon of the same frequency (two photons then exist: the original one, and the stimulated one).

The occurrence of (2) in preference to (3) or vice versa (the probability of an upward versus a downward transition) depends directly on the population of the two energy states involved. Normally, the lower energy state is most highly populated and the occurrence of (2) predominates over (3). However, in the laser, the population of the higher energy state is increased until it is larger than that of the lower (population inversion) by irradiation of the system with light of the proper frequency (pumping). Hence, in this case when the pumping radiation is removed, (3) predominates over (2).

As a practical example, the ruby laser will be considered. The ruby laser consists of a ruby crystal in the form of a rod. The ends of the rod are optically flat and parallel. The ruby material consists of an

aluminum-oxide (Al_2O_3) matrix which contains Cr^{3+} atoms as impurities. The Cr^{3+} atoms, referred to as the dopant, are responsible for the laser action. An energy level diagram for the ruby crystal in the optical region consists of three levels (rather than two as previously discussed) as shown below:



By exposing the ruby crystal to intense light with a frequency f_{31} , population inversion is achieved. Any atom raised to the E_1 energy state immediately undergoes a radiationless transition to energy state E_2 . E_2 is called a metastable energy state. A population inversion between E_1 and E_3 or E_2 and E_3 has a finite lifetime since these are nonequilibrium conditions. If energy is continually pumped into the system, it is possible to maintain a population inversion. (The transition between E_1 and E_2 results in thermal energy, and heating will occur.)

Note: The laser using three energy levels instead of two has an advantage in that it can be operated on a continuous basis. The two-level laser must have its pump power removed before it can operate, since it is pumping at the signal frequency. In the three-level laser, pumping occurs at the frequency which is not the signal frequency (f_{31}) hence pumping and

amplification (or oscillation) can go on simultaneously. The limiting factor on this continuous operation is the production of heat from the $E_1 - E_2$ transition and some form of cooling must be provided particularly at high power levels.

If, while the condition of population inversion exists, a photon of energy $h_{f_{23}}$ enters the system, the transition from energy state E_2 to E_3 occurs instantaneously. The two photons are now active in causing transitions in another pair of atoms thus producing four atoms, and the excess population in energy state E_2 is very rapidly dropped to the ground state E_3 . In order to enhance this effect, one end of the ruby rod is made perfectly reflecting while the other end is made partially reflecting so that some of the photons striking this end escape, and produce the laser beam, while the others continue to reflect back and forth between the ends causing further transitions. The ruby is cut to proper dimensions to act as a resonant cavity and the light escaping from the partially reflecting end is in phase and of almost pure frequency. The laser action, described above, occurs spontaneously since the population inversion has a finite lifetime and some finite time after pumping action has ceased, one atom must make the transition from E_2 to E_3 and provide the necessary photon of frequency f_{23} .

APPENDIX II

AREAS SUBTENDED BY LASER BEAMS

The laser beam is a radiation field which can be characterized by light rays diverging from a point source if the point of observation is a distance r from the crystal face such that

$$r > \frac{2D^2}{\lambda} \quad (\text{II-1})$$

where:

D = laser crystal diameter

λ = wavelength of laser.

At distances less than this, there exists the near field which has a much more involved configuration, and need not concern us here. For a ruby crystal having a diameter of 1 cm and a wavelength of 6943 Å, $r_{\min} = 2.8 \times 10^4$ cm. Even though this distance is large it will be used in the following discussion. Its value can be decreased by decreasing the diameter of the laser crystal and if optical path multiplication or folding is used, it should be possible to make a reasonably sized system.

If ϕ is the half-power beam angle, then from the Rayleigh Criterion:

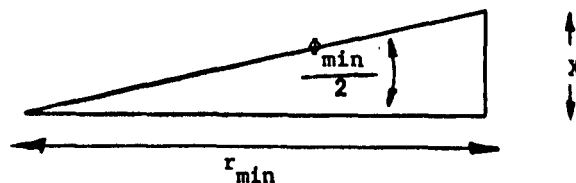
$$\phi_{\min} = 2.44 \lambda / D \quad (\text{II-2})$$

Using the same numbers as in the previous calculation:

$$\phi_{\min} = 1.6941 \times 10^{-4} \text{ radians}$$

$$\tan \frac{\phi_{\min}}{2} = \frac{X}{r_{\min}}$$

$$X = r_{\min} \tan \frac{\phi_{\min}}{2} = r_{\min} \frac{\phi_{\min}}{2} \quad [(\text{Since } \phi_{\min}/2 \text{ small})]$$



The area subtended at r_{\min} is given by πX^2 .

$$A_{r_{\min}} = \pi X^2 = \pi r^2 \frac{\phi_{\min}^2}{4} = (3.14)(7.84)(7.17)(10^{-9})(10^8) \\ = 17.6 \text{ cm}^2$$

and the area subtended is a circle of radius 2.37 cm.

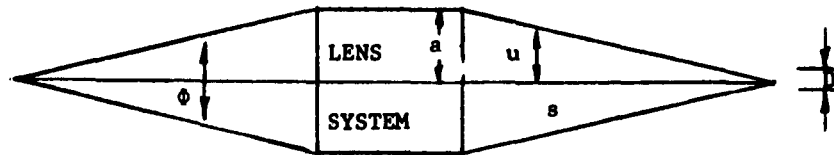
If the beam is caused to enter a lens system placed at a distance r_{\min} from the laser, the output of the lens system will have a circular image of diameter given by

$$D = \frac{1.22 \lambda}{n \sin u} \quad (\text{II-3})$$

where:

u = image angle

n = index of refraction of medium into which the light is emerging.



The expression $n \sin u$ is called the numerical aperture of the lens system. From the diagram it is seen that:

$$\tan u = a/s$$

If $u = 0^\circ$, then a must equal zero.

If $u = 90^\circ$ $\tan u \rightarrow \infty$, and for a fixed distance s , $a \rightarrow \infty$ which implies a lens system of infinite radius.

Hence, in all practical cases,

$$0^\circ < u < 90^\circ$$

$$0 < \sin u < 1$$

For reasonable values of $\sin u$ and $n = 1$, D approximates the wavelength of the light used or several times this value. If λ is $6,943 \text{ \AA}$, then D is approximately the same or $.6943 \mu$ which is smaller than any ordinary cell and approximates the diameter of the nucleus of many cells.